[redacted]

INVOICE CHECK LIST

MKULTRA Subproject

[redacted]

At Date of Original Authorization	Period Covered	Time Extended To	Allotment Number	Amount of Obligation
12 July 1961	1 year		2121-[redacted]	9,360.00

Additional Authorizations	Period Covered	Time Extended To	Allotment Number	Amount of Obligation
#3	1 SEP 63	31 AUG 64	4125-1390-3902	9,360.00

Invoice Number	Date	Amount	Balance
[redacted]	12 July 1961	#9,360.00 ✓	-0-
Auth #2	24 July 1962	9360.00	9,360.00
2	5 Oct 1962	9360.00 ✓	-0-
AUTHORIZATION #3	29 JULY 1963	9,360.00	9,360.00
Invoice #3	1 Aug 63	9,360.00 ✓	-0-
REMARKS:			

[redacted]

133-1

[redacted]

No. 89

Cost Account 4125-1390-3902

Object Class

Date	Remarks and References	Obligations Incurred	Obligations Liquidated	Unliquidated Balance
30 JUL 1963	MKULTRA Sub Proj. 133 7/29/63	9,360.00		9,360.00
15 AUG 1963	Invoice #3		9,360.00	

[redacted]

133-2

[redacted]

Date: 29 July 1963

MEMORANDUM FOR: C/TSD/FASS

SUBJECT : MKULTRA, Subproject No. 133

Under the authority granted in the memorandum dated [illegible] from the DCI to the DD/A, and the extension of this authority in [illegible] memoranda, Subproject 133 has been approved, and $9,360.00 of the over-all Project MKULTRA funds have been obligated to [illegible] subproject's expenses and should be charged to cost center 4125-1390-3902

[redacted] A

Concur:

[redacted] A

for Asst. Chief, TSD for R&D [redacted]

[redacted] A

Chief, Sci. TSD/R&D

Concur:

Corrected XC - $4,680
XD - 4,680

APPROVED FOR OBLIGATION OF FUNDS:

I CERTIFY THAT FUNDS ARE AVAILABLE
OBLIGATION AUTHORIZED $9
CHARGE TO ALLOTMENT NO. 4125-1390-3902

AUTHORIZING OFFICIAL

Chief, DD/P/TSD

Date:

Distribution:
Orig & 1 - Addressee
2 - TSD/BF

[redacted]

[redacted]

133-3

(When Filled In)

REQUEST FOR ADVANCE OF FUNDS	NAME OF ADVANCEE: MKULTRA # 133	DATE: 14 August 1963		AMOUNT: [illegible]0.00 Fee $[illegible],00[illegible].00	VOUCHER NO. (Finance use only)
	PAYABLE TO: See attached payment inst[redacted]	ROOM	BUILDING	TELEPHONE EXT.	G

PURPOSE

Advance to MKULTRA Subproject # 133, invoice # 3 for activity approved by C/TSD on 29 July 1963. Accounting for this advance will be in accordance with attachment ~~F~~ 8 of MKULTRA Fiscal Annex.

Pls forward checks (2) to TSD/BB thru TSD/Budget Office no later than 26 August 1963

STATUS OF OUTSTANDING ADVANCES

DATE	AMOUNT	UNACCOUNTED BALANCE

I agree that I will fully account for this advance by submission of vouchers and refund of any unexpended balance to the reporting point stated and by the due date checked below. In the event of my failure to so account and refund any unexpended balance, I authorize deduction from my salary to effect settlement.

REPORTING POINT	DUE DATE	✓
FINANCE DIVISION - HEADQUARTERS	025	✓
	ON ARRIVAL AT DESTINATION ON OR ABOUT	
	MONTHLY - ON THE LAST WORKDAY OF EACH MONTH	

REQUESTING OFFICER

DATE: 14 Aug. 1963 — SIGNATURE: [redacted] A

I CERTIFY FUNDS ARE AVAILABLE

OBLIGATION REFERENCE NO.: 89 — CHARGE ALLOTMENT NO.: 4125-1390-3902

DATE: 14 AUG 1963 — SIGNATURE:

APPROVED

DATE: 8/15/63 — SIGNATURE OF APPROVING OFFICER: SIDNEY GOTTLIEB, DC/TSD

CERTIFIED FOR PAYMENT OR CREDIT

DATE: — AUTHORIZED CERTIFYING OFFICER:

RECEIPT FOR FUNDS ADVANCED

I acknowledge receipt of funds in the amount stated hereunder to be used for the purpose stated and accounted for as shown above.

DATE: — AMOUNT:

SIGNATURE OF ADVANCEE:

SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION

(PREP)ARED BY: — REVIEWED BY: — VOUCHER NO. 7-12

DESCRIPTION-ADVANCE ACCOUNTS 13-27	28-33 T/A NO. / P.O. NO. / 32-33 DIV.	34-39 STATION CODE / PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / FY	43 FUNDS	45-46 PAY PER LIO CODE	47-52 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	68-70 DUE DATE / OBJECT CLASS	71-80 AMOUNT DEBIT	CREDIT
						810		2971	6 [redacted] 9000.00			
						010		2970				

CHECK# ______ IN THE AMOUNT OF $______ RECEIVED.

[redacted]

TOTALS

1A

FORM 5-60 281 USE PREVIOUS EDITIONS. (38-49)

133-4

[redacted]

No. 176

Cost Account 3125-1390-3902

Object Class

Date	Remarks and References	Obligations Incurred	Obligations Liquidated	Unliquidated Balance
26 JUL 1962	MKULTRA, Sub-proj 133	9,360.00		9,360.00
9 OCT 1962	Inv # 2		9360.00	

[redacted]

[redacted]

Date: [illegible] July 1962

MEMORANDUM FOR: The Comptroller

ATTENTION : Finance Division

SUBJECT : MKULTRA, Subproject 133

Under the authority granted in the memorandum dated 13 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 133 has been approved and [illegible].00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to such number [illegible]-1390-390[illegible]

[redacted] A
Chief,
TSD/Biological Branch

APPROVED FOR OBLIGATION
OF FUNDS:
Original signed by
Sidney Gottlieb
ACTING CHIEF, TSD

Date: 25 JUL 1962

Distribution:
Original & 2 - Addressee
✓1 - TSD FASS BB Category IVc
2 - TSD BB

I CERTIFY THAT FUNDS ARE
OBLIGATION REFERENCE NO. 176
CHARGE TO ALLOTMENT NO. 3125-1390-3902-

AUTHORIZING OFFICER

[redacted]

133-10

REQUEST FOR ADVANCE OF FUNDS

NAME [redacted] H | DATE 5 October 1962 | AMOUNT 9,000.00 360.00 (Fee) | VOUCHER NO. (Finance use only)

PAYABLE TO See attached. | ROOM [redacted] | BUILDING | TELEPHONE EXT. | C

PURPOSE

To [redacted] H for funding MKULTRA Subproject 133 Invoice #2 which activity was approved by C/TSD on 25 July 1962. Accounting to be in accordance with the Fiscal Annex Attachment C.

STATUS OF OUTSTANDING ADVANCES

DATE	AMOUNT	UNACCOUNTED BALANCE

I agree that I will fully account for this advance by submission of vouchers and refund of any unexpended balance to the reporting point stated and by the due date checked below. In the event of my failure to so account and refund any unexpended balance, I authorize deduction from my salary to effect settlement.

REPORTING POINT

FINANCE DIVISION - HEADQUARTERS

DUE DATE

	✓
ON ARRIVAL AT DESTINATION	
ON OR ABOUT	
MONTHLY - ON THE LAST WORKDAY OF EACH MONTH	

REQUESTING OFFICER

DATE | SIGNATURE [redacted] A

I CERTIFY FUNDS ARE AVAILABLE

OBLIGATION REFERENCE NO. 176 | CHARGE-ALLOTMENT NO. 3125-1390-3902

DATE 9 OCT 1962 | SIGNATURE

APPROVED

DATE | SIGNATURE OF APPROVING OFFICER: Sidney Gottlieb, DC/TSD

CERTIFIED FOR PAYMENT OR CREDIT

DATE | AUTHORIZED CERTIFYING OFFICER

RECEIPT FOR FUNDS ADVANCED

I acknowledge receipt of funds in the amount stated hereunder to be used for the purpose stated and accounted for as shown above.

DATE | AMOUNT

SIGNATURE OF ADVANCEE

SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION

PREPARED BY

REVIEWED BY

VOUCHER NO. 7-12

DESCRIPTION-ALL OTHER ACCOUNTS 13-33 / DESCRIPTION-ADVANCE ACCOUNTS 13-27	28-33 T/A NO. / P.O. NO. / 32-33 DIV.	34-39 STATION CODE / PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / IFY	43 FUNDS	45-46 PAY PERIOD CODE	47-52 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	62-67 CK. NO. / X REF. NO.	68-70 DUE DATE / OBJECT CLASS	71-80 AMOUNT DEBIT	71-80 AMOUNT CREDIT
						0069284						9000.00	
						0069286						360.00	
						CHECK #___ IN THE AMOUNT OF $___ RECEIVED							
						A [redacted]							12 Oct 62
											TOTALS		

FORM 5-60 281 USE PREVIOUS EDITIONS. (38-49)

133-7

[redacted]

No. 163

Cost Account 2125-1390-5902

Object Class

Date	Remarks and References	Obligations Incurred	Obligations Liquidated	Unliquidated Balance
24 JUL 1961	Mobilize Sub 133	9,360.00		9,360.00
2 AUG 1961	Inv #1		9,360.00	-

[redacted]

133-8

[redacted]

DATE: 25 May 1961

MEMORANDUM FOR : THE COMPTROLLER

ATTENTION : Finance Division

SUBJECT : MKULTRA, Subproject 133

Under the authority granted in the memorandum dated 13 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 133 has been approved, and $9,360.00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 2125-1390-3582.

A [redacted]
Chief
TSD/Research Branch

APPROVED FOR OBLIGATION OF FUNDS:

Original signed by [redacted] A

Research Director

Date: 12 May 61

I CERTIFY THAT FUNDS ARE AVAILABLE:
OBLIGATION REFERENCE No. 140
CHARGE TO ALLOTMENT No. 2125-1390-3582

AUTHORIZING OFFICER

Distribution:
Orig. & 2 - Addressee
1 - TSD/FACS
2 - TSD/RB (1 chron, 1 file(finance)

[redacted]

133-9

[redacted]

12 July 1961

MEMORANDUM FOR: Chief, Finance Division

VIA : TSD/Budget Officer

SUBJECT : MKULTRA, Subproject 133, Invoice #1
Allotment 2125-1390-3902

1. Invoice No. 1 covering the above subproject is attached. Payment should be made as follows:

Cashier's check in the amount of $9,000.00 drawn on a [redacted] payable to [redacted] B

Cashier's check in the amount of $360.00 drawn on a [redacted] payable to [redacted] B

2. Please forward the checks to Chief, TSD/Research Branch, through TSD/Budget Officer, no later than 24 July 1961.

3. This is a final invoice. However, since it is anticipated that additional funds will be obligated for this project, the files should not be closed.

[redacted] A
Chief
TSD/Research Branch

E [redacted]
CHECK 2-064674 ... AMOUNT OF $9,000
RECEIVED [redacted] 14 Aug 61

Attached:
Invoice & Certifications

Distribution:
Orig & 2 - Addressee

E [redacted] 201154 ... 360
REC...
A [redacted] 14 Aug 61

8033

[redacted]

CONFIDENTIAL FUNDS POSTING VOUCHER

VOUCHER NO. 7-12 | DATE 2-6 | VOUCHER NO. 7-12

DESCRIPTION-ALL OTHER ACCOUNTS 13-33 / DESCRIPTION-ADVANCE ACCOUNTS 13-27	28-33 T/A NO. / P.O. NO. / 32-33 DIV.	34-39 STATION CODE / PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / FY	43 FUNDS	45-46 PAY PER. LIQ. CODE	47-52 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	62-67 CK. NO. / X REF. NO.	68-70 DUE DATE / OBJECT CLASS	71-80 AMOUNT DEBIT	71-80 AMOUNT CREDIT
Subproj 133 Inv't			8821			140		6010	25-1390-	3402	752	9360.00	
MK ULTRA						137		1100		1176			9000.00
"						108		1100		378			360.00
EXPLANATION OF ENTRY										TOTALS			

See attached

9,000.00
2-064674 360.00
26654

DATE	PREPARED BY	DATE	REVIEWED BY	CERTIFIED FOR PAYMENT OR CREDIT
8/8/61	[redacted] A			DATE / SIGNATURE OF CERTIFYING OFFICER

FORM 10-59 606 USE PREVIOUS EDITIONS. [redacted] (10-49)

(When Filled In)

ACCOUNTING BY INDIVIDUAL FOR ADVANCE

NOTE: Follow Instructions on Reverse

SUBMITTED BY: MKULTRA Sub-project # 133

PERIOD OF ACCOUNTING: FROM 1 September 196[illegible] TO 30 April 1965

DATE - VOUCHER NO. 2-12

1. CASH ON HAND BEGINNING OF PERIOD	$ 307.88	
2. OUTSTANDING ADVANCES BEGINNING OF PERIOD		
3. RECEIPTS THIS PERIOD:		

RECEIPT NUMBER	DATE	DESCRIPTION

4. TOTAL TO ACCOUNT FOR	$ 307.88

5. EXPENSES THIS PERIOD:

VOUCHER NUMBER	DATE	DESCRIPTION	AMOUNT
		Expenses - See attached certification	$ 307.88
		TOTAL EXPENSES	$ 307.88

6. REFUNDED HEREWITH ( ) CASH ( ) CHECK ( ) MONEY ORDER	
7. OUTSTANDING ADVANCES END OF PERIOD (Attach listing)	
8. CASH ON HAND END OF PERIOD OR BALANCE DUE ADVANCEE	
9. TOTAL ACCOUNTED FOR	$ 307.88

I CERTIFY FUNDS ARE AVAILABLE

OBLIG. REF. NO.: 89

CHARGE FAN ACCOUNT NO.: 4125-1390-3902

DATE:

SIGNATURE OF AUTHORIZING OFFICER:

APPROVED

DATE:

SIGNATURE OF APPROVING OFFICER: SIDNEY GOTTLIEB C/TSD

CERTIFIED FOR PAYMENT OR CREDIT

DATE:

SIGNATURE OF CERTIFYING OFFICER:

I certify that the expenditures listed hereon and on any attachments were incurred for official purposes, of a confidential nature, that payment or credit therefor has not been received, and that this accounting is true and correct.

SIGNATURE OF PAYEE: [redacted] C/TSD/[redacted]

SPACE BELOW FOR EXCLUSIVE USE OF OFFICE OF FINANCE

DESCRIPTION - ALL OTHER ACCOUNTS 13-33 / DESCRIPTION - ADVANCE ACCOUNTS 13-27	28-33 T/A NO. / SHIP. DOC. NO. / 32-33 DIV.	34-39 STATION CODE / REC. RPT. NO. / PROJECT NO.	40-41 EXPEND CODE	42-47 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	48-49 PAY PER. LIO. CODE	50 CAR COST YR.	51-54 GENERAL LEDGER ACCT. NO.	55-66 COST - FAN ACCOUNT SYMBOL (FUNDS)	61-66 CK. NO. / X REF. NO.	67-70 OBJECT CLASS / 68-70 DUE DATE	71-80 AMOUNT DEBIT	71-80 AMOUNT CREDIT
Inv 3 Mar 89	25	770185		133			760.0					307.88
MKU Inv 3							760.0				307.88	

DATE: 7/Mar. 1965

PREPARED BY: [redacted] A

DATE:

REVIEWED BY:

TOTALS: 307.88 | 307.88

FORM 4-65 282 OBSOLETE PREVIOUS EDITIONS

(1-49)

133-12

[redacted]

CERTIFICATION

This is to certify that I have received an accounting from MKULTRA Sub-project # 133 for the period 1 September 1964 - 30 April 1965. The accounting reflects expenses in the amount of $307.88 for the remaining grant balance.

The accounting statement and payment certification will be retained by TSD and will be made available for review in TSD if necessary.

I certify that satisfactory services represented by the accounting have been received and that to the best of my knowledge and belief the funds expended were for the purposes authorized by the project approval.

[redacted]
Chief, TSD/BB

[redacted]

[redacted]

	9/1/62-8/31/64	9/1/64-4/30/65	Total
Salaries			
Previous Report	$12,882.59		$12,882.59
Total Salaries	$12,882.59		$12,882.59
Supplies and Expenses			
Previous Report	2,636.81		2,636.81
[redacted]		58.48	58.48
		132.11	132.11
		88.54	88.54
		(9.55)	(9.55)
		(.50)	(.50)
Petty Cash		.69	.69
Total Expenses	2,636.81	269.77	2,906.58
Total Direct Costs	15,519.40	269.77	15,789.17
Indirect Costs -			
14% of Direct Costs	2,172.71	37.77	2,210.48
Total	17,692.11	307.54	17,999.65
Funds Provided			18,000.00
Balance (Written Off)			.35

I certify that services or materials have been satisfactorily received and the expenditures were incurred on official business.

[redacted]

Date: 10 March 1966

133-14

[redacted]

12 October 1964

[redacted]

This is to acknowledge the extension of the expiration date of [redacted] account no.) from the [redacted] Word of the extension was transmitted to me via telephone by [redacted] on 12 October 1964.

The current balance on account 440.11 is $307.88. This sum will be used for payment of publication costs of a paper in ECONOMIC GEOLOGY and the remainder for the purchase of expendable laboratory items.

Thank you for permitting this [redacted] extension.

Sincerely yours,

[redacted]

cc Research Division
Comptroller

#133

133-15

[redacted]

[redacted]

October 12, 1964

[redacted]

[redacted]

In response to your letter of October 8, attached please find an accounting of [redacted] funds provided for research performed under the direction of [redacted] for the period beginning September 1, 1962 and ending August 31, 1964.

We trust that the report is sufficiently detailed for your purposes.

Please advise what disposition should be made of the unexpended balance of $307.88.

Very truly yours,

[redacted]

Assistant to the Comptroller

[redacted]

enclosure

c.c. [redacted]

[redacted] Assoc. Professor	$1,746.11	$1,955.16	$3,703.27
Lab. Assistant	3,958.72	4,362.60	8,321.32
Lab. Technician	-	858.00	858.00
Total Salaries	$5,706.83	$7,175.76	$12,882.59
Supplies & Expenses			
[redacted]	$1,055.92	$729.57	$1,785.49
Photographic Service	47.24	7.50	54.74
Institute Stores	17.56	12.78	30.34
Telephone Expense	19.75	11.50	31.25
Central Duplicating	1.75	-	1.75
Petty Cash	3.60	15.80	19.40
[redacted]	31.70	-	31.70
Library charges	14.46	1.73	16.19
[redacted]	13.05	-	13.05
	5.00	9.55	14.55
	4.30	-	4.30
	5.75	-	5.75
	121.06	101.15	222.21
	26.89	-	26.89
Laboratory Supplies	5.70	1.70	7.40
[redacted]	10.07	239.33	249.40
Electronic Stores	-	.84	.84
[redacted]	-	46.36	46.36
	-	36.85	36.85
	-	21.47	21.47
	-	14.50	14.50
Xerox Service	-	2.38	2.38
Total Expenses	$1,383.80	$1,253.01	$2,636.81
Total Direct Costs	$7,090.63	$8,428.77	$15,519.40
Indirect Cost - 14% of Direct Cost	$992.69	$1,180.03	$2,172.72
Total	$8,083.32	$9,608.80	$17,692.12
Funds Provided	$9,000.00	$9,000.00	$18,000.00
(Overexpended) Underexpended	$916.68	($608.80)	$307.88

133-16

[redacted]

12 October 1964

[redacted]

This is to acknowledge the extension of the expiration date of [redacted] account no.) from the [redacted] Word of the extension was transmitted to me via telephone by [redacted] on 12 October 1964.

The current balance on account 440.11 is $307.88. This sum will be used for payment of publication costs of a paper in ECONOMIC GEOLOGY and the remainder for the purchase of expendable laboratory items.

Thank you for permitting this [redacted] extension.

Sincerely yours,

[redacted]

cc Research Division
Comptroller

#133

133-17

[redacted]

19 October 1964

MEMORANDUM FOR : Chief, Covert Claims Branch

SUBJECT : MKULTRA Subproject 133

Attached are certifications from Chief, Biological Branch, for MKULTRA Subproject 133 Invoices 2 and 3 and should be recorded in account 760.

/s/ 19 OCT 1964

[redacted]
C/TSD/SS

Distribution:
Original & 1 - Addressee

[redacted]

[redacted]

133-18

[redacted]

October 12, 1964

[redacted]

In response to your letter of October 8, attached please find an accounting of [redacted] funds provided for research performed under the direction of [redacted] for the period beginning September 1, 1962 and ending August 31, 1964.

We trust that the report is sufficiently detailed for your purposes.

Please advise what disposition should be made of the unexpended balance of $307.88.

Very truly yours

[redacted]

Assistant to the Comptroller

[redacted]

enclosure

[redacted]

133-18-138

Microbiology of Minerals

	9/1/62 - 8/31/63	9/1/63 - 8/31/64	Total
Salaries			
[redacted] Assoc. Professor	$1,748.11	$1,955.16	$3,703.27
Lab. Assistant	3,958.72	4,362.60	8,321.32
Lab. Technician	-	858.00	858.00
Total Salaries	$5,706.83	$7,175.76	$12,882.59
Supplies & Expenses			
[redacted]	$1,055.92	$729.57	$1,785.49
Photographic Service	47.24	7.50	54.74
Institute Stores	17.56	12.78	30.34
Telephone Expense	19.75	11.50	31.25
Central Duplicating	1.75	-	1.75
Petty Cash	3.60	15.80	19.40
[redacted]	31.70	-	31.70
Library charges	14.46	1.73	16.19
[redacted]	13.05	-	13.05
[redacted]	5.00	9.55	14.55
[redacted]	4.30	-	4.30
[redacted]	5.75	-	5.75
[redacted]	121.06	101.15	222.21
[redacted]	26.89	-	26.89
Laboratory Supplies	5.70	1.70	7.40
[redacted]	10.07	239.33	249.40
Electronic Stores	-	.84	.84
Economic Geology Pub.	-	46.36	46.36
[redacted]	-	36.85	36.85
[redacted]	-	21.47	21.47
[redacted]	-	14.50	14.50
Xerox Service	-	2.38	2.38
Total Expenses	$1,383.80	$1,253.01	$2,636.81
Total Direct Costs	$7,090.63	$8,428.77	$15,519.40
Indirect Cost - 14% of Direct Cost	$992.69	$1,180.03	$2,172.72
Total	$8,083.32	$9,608.80	$17,692.12
Funds Provided	$9,000.00 INV. 2	$9,000.00 INV. 3	$18,000.00
(Overexpended) Underexpended	$916.68	($608.80)	$307.88

I certify that services or materials have been satisfactorily received and the expenditures were incurred on official business.

[redacted]

Date

[redacted] TO BE GIVEN EXTENTION OF TIME TO SPEND THIS SMALL RESIDUE PER [redacted] 10-16-64

133-9

ACCOUNTING BY INDIVIDUAL FOR ADVANCE

NOTE: *Follow Instructions on Reverse*

SUBMITTED BY: MKULTRA Sub 133

PERIOD OF ACCOUNTING: FROM 1 October 1961 TO 30 November 1962

VOUCHER NO. *(Finance use only)*: 451861

RECEIPTS

			$
1. CASH ON HAND BEGINNING OF PERIOD			
1a.			
2. RECEIPT NUMBER	DATE	DESCRIPTION	
	12 Jul 1961	MKULTRA Advance Invoice # 1 [redacted]	8,500.00
TOTAL TO ACCOUNT FOR			$8,500.00

DISBURSEMENTS

3. VOUCHER NUMBER	DATE	DESCRIPTION	AMOUNT $
		See Attached Certification	
		TOTAL EXPENSES	$ 8,500.00
3a.			
4. REFUNDED HEREWITH		CASH / CHECK / MONEY ORDER	
5. CASH ON HAND END OF PERIOD			
		TOTAL ACCOUNTED FOR	$ 8,500.00

I CERTIFY FUNDS ARE AVAILABLE

OBLIGATION REFERENCE NO.: 140

CHARGE ALLOTMENT NO.: 2125-1390-3902

DATE:

APPROVED

SIGNATURE OF APPROVING OFFICER: SIDNEY GOTTLIEB, DC/TSD

I certify that the expenditures listed hereon and on any attachments were incurred for official purposes of a confidential nature, that payment or credit therefor has not been received, and that this accounting is true and correct.

DATE:

SIGNATURE OF AUTHORIZING OFFICER:

CERTIFIED FOR PAYMENT OR CREDIT

DATE:

SIGNATURE OF CERTIFYING OFFICER:

SIGNATURE OF PAYEE: [redacted]

SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION

PREPARED BY:

REVIEWED BY:

VOUCHER NO. 7-12

DESCRIPTION - ALL OTHER ACCOUNTS 13-33	28-33 T/A NO. P.O. NO. / DIV.	34-39 STATION CODE PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / FY	43 FUNDS	45-46 PAY PER. LIQ. CODE	47-52 OBLIG. REF. NO. ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	62-67 CK. NO. / X REF. NO.	68-70 DUE DATE OBJECT CLASS	71-80 AMOUNT DEBIT	AMOUNT CREDIT
DESCRIPTION - ADVANCE ACCOUNTS 13-27													
										TOTALS			

FORM 8-61 282 USE PREVIOUS EDITIONS.

(1)

133-20

TRANSMITTAL SLIP	DATE

TO: C/TSD/SS

ROOM NO.	BUILDING

REMARKS:

ORIGINAL AUTHORIZATION FOR SUB 133 WAS FOR $9,000. HOWEVER, ONLY $8,500 PASSED BY [redacted]H (THE REMAINING $500 IS PART OF $2914.93 STILL TO BE RETURNED BY [redacted]H THIS ACCOUNTING WITH $160.39 ADDED FOR PUBLICATION AND REPORT CHARGES CLEARS UP THE $8500 UNDER INVOICE 1.

FROM: [redacted] A

ROOM NO.	BUILDING	EXTENSION

FORM NO. 241 1 FEB 55 REPLACES FORM 36-8 WHICH MAY BE USED. ☆ GPO: 1952—O-435445 (47)

133-21

[redacted] B

[redacted] B

[redacted] B

December 18, 1962

[redacted] B

Dear Sir:

Enclosed please find our first fiscal report for [redacted] C

[redacted] C, for the period 10/1/61 - 11/30/62.

Very truly yours,

[redacted] B

Assistant to the Comptroller

[redacted] B

Enclosure

133-21

44006

[redacted] B

[redacted] B

Interim Report #1
Period 10/1/61 - 11/30/62

Salaries & Wages	$4,673.85
Supplies & Expenses	2,435.12
Travel	194.48
Other Costs - Reprints	12.00
	$7,315.45
Indirect Costs 14%	1,024.16
Total	$8,339.61
	160.39
	8,500.00

$160.39 - Pay [redacted] B for [publication and reprint charges.]

I have examined and approved the submitted expenditures.

[redacted] A
Chief
TSS/Chemical Div

Date: 2/7/63

133-22

[redacted]MKULTRA

[redacted]

PROJECT CRYPTO : MKULTRA # 133

[redacted]

PRINCIPAL INVESTIGATOR : [redacted]

DATE RENEWED : 29 July 1963

During the previous year [redacted] concentrated his efforts on the elucidation of mechanisms of microbial action on mineral sulfides and manganese nodules.

Bacterial dissolution of As_2S_3, FeS_2FeAs_2, and $3Cu_2SAs_2S_5$ was demonstrated. The end products were shown to be arsenite, arsenate, Fe^{++}, Fe^{+++}, and Cu^{++}, with the fate of the sulfur still undetermined.

Studies on CuS_2 indicated that Thiobacillus sp. were unable to solubilize this mineral in pure culture. They found that dissolution of this mineral under natural conditions necessitated the presence of protozoa and fungi. These organisms were all active under conditions of copper concentration which would normally be lethal.

Samples of manganese nodules collected by [redacted] in the Atlantic Ocean (as a guest of a [redacted]) were studied to determine the nature of possible bacterial transformations. A preliminary scheme for this activity was determined to be a non-biological dismutation Mn^{++} and MnO_2 forming Mn^{+++}. This latter ion must be stabilized and it is likely that organic by-products of bacterial metabolism play this role.

[redacted] has furnished three cultures with interesting characteristics for application to the power source studies under MKULTRA # 78.

During the present year methods for mass culture of mineral oxidizers have been developed which will furnish cell material for enzymatic and resting cell studies. Continued work on manganese transformations is emphasizing environmental effects.

[redacted]

[redacted]MKULTRA

133-22
REVISED

Date 5 September 1963

Branch BB Category NEW MATERIALS & CONCEPTS (X)
(formerly IVc)

Project Title Microbial transformations of Minerals Item Classification Unclassified (Reports)

Project Crypto MKULTRA 133 Crypto Classification Unclassified

Branch Project No. N.A. Project Engineer A [redacted]

Contractor [redacted] C

Contract No. N.A. Task No. N.A.

Type of Contract MKULTRA Date Initiated 12 July 1963

Cost $9,360.00 Completion Date Continuing

Purpose: To provide the services of [redacted] C and to support research on fundamental mechanisms of mineral transformation fy microorganisms. In addition to the direct potential for new energy source systems, the contract may provide by-products useful for material deterioration application.

Status: Current and satisfactory.

Requirement: Internally generated in support of the requirements to seek new and better sources of energy for battery application.

133-23

RECEIPT

Receipt is hereby acknowledged of [redacted] Check No. 0102970, dated 23 August 1963, drawn on the [redacted] payable to [redacted] in the amount of $360.00.

Receipt is hereby acknowledged of [redacted] Check No. 0102971, dated 23 August 1963, drawn on the [redacted] payable to [redacted] in the amount of $9,000.00.

NAME [redacted]

DATE Sept 10 1963

INV 3

133-24

REQUEST FOR ADVANCE OF FUNDS

NAME OF ADVANCEE	DATE	AMOUNT (U.S. Fee)	VOUCHER NO. (Finance use only)
MKULTRA # 133	14 August 1963	$ [redacted]	
PAYABLE TO	**ROOM**	**BUILDING**	**TELEPHONE EXT.**
See attached payment inst.	1117	Westcott	2822

PURPOSE

Advance to MKULTRA Subproject # 133, invoice # 3 for activity approved by C/TSD on 27 July 1963. Accounting for this advance will be in accordance with attachment A of MKULTRA Fiscal Annex.

Pls forward checks (2) to TSD/RB Mark TSD/Budget Office no later than 26 August 1963

STATUS OF OUTSTANDING ADVANCES

DATE	AMOUNT	UNACCOUNTED BALANCE

I agree that I will fully account for this advance by submission of vouchers and refund of any unexpended balance to the reporting point stated and by the due date checked below. In the event of my failure to so account and refund any unexpended balance, I authorize deduction from my salary to effect settlement.

REPORTING POINT

FINANCE DIVISION - HEADQUARTERS

DUE DATE ✓

- ON ARRIVAL AT DESTINATION ON OR ABOUT
- MONTHLY - ON THE LAST WORKDAY OF EACH MONTH

REQUESTING OFFICER

DATE: 14 Aug. 1963 SIGNATURE: [redacted] A

APPROVED

DATE:

SIGNATURE OF APPROVING OFFICER: SIDNEY GOTTLIEB, IC/TSD

RECEIPT FOR FUNDS ADVANCED

I acknowledge receipt of funds in the amount stated hereunder to be used for the purpose stated and accounted for as shown above.

DATE: AMOUNT:

SIGNATURE OF ADVANCEE:

I CERTIFY FUNDS ARE AVAILABLE

OBLIGATION REFERENCE NO.:

CHARGE ALLOTMENT NO.: 4125-1300-3902

DATE: SIGNATURE:

CERTIFIED FOR PAYMENT OR CREDIT

DATE: AUTHORIZED CERTIFYING OFFICER:

SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION

PREPARED BY: REVIEWED BY: VOUCHER NO. 7-12

DESCRIPTION-ADVANCE ACCOUNTS 13-27	DESCRIPTION-ALL OTHER ACCOUNTS 13-33	28-33 T/A NO. / P.O. NO. / 32-33 DIV.	34-39 STATION CODE / PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / FY	43 FUNDS	45-46 PAY PERIOD CODE	47-52 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	62-67 CK. NO. / X REF. NO.	68-70 DUE DATE / OBJECT CLASS	71-80 AMOUNT DEBIT	71-80 AMOUNT CREDIT
											TOTALS			

FORM 5-60 281 USE PREVIOUS EDITIONS. (38-49)

133-25

29 July 1963

133

133 $9,360.00

4125-1390-3902

[redacted] A

[redacted] A

Chief, Sci. TSD/R&D

C

for [redacted] R&D 30 JUL 1963

C

[redacted] 30 JUL 1963

I CERTIFY THAT FUNDS ARE AVAILABLE:
OBLIGATION REFERENCE NO. [redacted] 3403
CHARGE TO ALLOT[redacted]

[redacted] A

Sidney Gottlieb
for
July 30, 1963

133-26

[redacted]

Date 29 July 1963

Branch BB Category New Materials & Concepts (X)

Project Title Microbial transformations of Minerals Item Classification Unclassified (Reports)

Project Crypto MKULTRA #133 Crypto Classification Unclassified

Branch Project No. N.A. Project Engineer [redacted] A

Contractor [redacted] C

Contract No. N.A. Task No. N.A.

Type of Contract MKULTRA Date Initiated 12 July 1963

Cost $9,320.00 $9,000.00 Completion Date Continuing

Purpose: To provide the services of [redacted] C and to support research on fundamental mechanisms of mineral transformation by microorganisms. In addition to the direct potential for new energy source systems, the contract may provide by-products useful for material deterioration application.

Status: Current and satisfactory.

REQUIREMENT SOURCE: Internally generated in support of the requirements to seek new and better sources of energy for battery application.

[redacted]

[redacted]

133-27

[redacted]

DRAFT

29 July 1963

MEMORANDUM FOR : THE RECORD

SUBJECT : Continuation of MKULTRA, Subproject #133

1. The purpose of MKULTRA, Subproject 133 is to enable TSD/BB to utilize the services of [redacted]

2. [redacted] continues to provide data and materials which lend themselves to new and unique approaches to energy production and transformation (bio-batteries). His studies on basic mechanisms of mineral transformations also provide new potential paths for deterioration of metals.

3. [redacted] will function as [redacted] and [redacted] during this fiscal year. The cost of this program for one year will be $9,000.00 to which must be added $360.00 which represents a 4% service charge to be retained by the [redacted] The total cost of the program will not exceed $9,360.00. Charges should be made against Allotment No. 4125-1390-3902.

4. It is not anticipated that permanent equipment other than that listed in the budget will be required for this program. Title to the equipment listed will be retained by the [redacted] in lieu of higher overhead rates.

[redacted]

133-27

[redacted]

5. Documentation and accounting for travel expenses which are reimbursable by [redacted] will conform to the accepted practice of that organization.

6. [redacted] has been cleared COVERTLY and is unwitting and will remain unwitting of the true nature of the sponsor.

[redacted]
TSD/Biological Branch

[redacted]
Chief
TSD/Biological Branch

[redacted]
Chief, Sci. TSD/R&D

Attachment:
Proposal

Distribution:
Original only

[redacted]

133-27
Signed Copy

[redacted] B

Proposal No. [redacted] C

entitled

[redacted] C

for renewal of [redacted] B

for the period

1 September 1963 - 31 August 1964

Submitted on behalf of

[redacted] C/B

April 1963

PURPOSE OF STUDY

This proposal is a request for renewal for the period 1 September 1963-31 August 1964 of the grant-in-aid presently supporting research on [redacted] The field of investigation covered by the present grant is so broad and relatively unexplored that several additional years of research can be profitably spent in unraveling the problem. The results of the research carry increasing significance not alone academically but also practically, as in public health, conservation of mineral resources, mineral exploration, and mineral extraction. In public health, these investigations are providing clues to the possible origin and control of acid ferruginous, and/or acid cupriferous, and/or acid arsenical stream pollution, deriving from bacterial action on natural mineral deposits in certain geographical locations[1]. With continued population increase, maintenance of all water resources in a condition fit for human consumption becomes more and more important. The work is also providing additional clues to the understanding of manganese biochemistry in public water supplies, where manganese together with iron can cause discoloration of water and water containers, and where manganese and iron are deposited biologically in water mains and pipes. In the area of conservation of mineral resources, the work under [redacted] provides a basis for assessing and controlling any relative instability of mineral deposits in relation to microbes. It seems evident that uncontrolled bacterial leaching could conceivably lead to depletion and eventual complete translocation of some deposits. In mineral exploration, this work indicates that special groups of microorganisms known to be able to live at the expense of a specific mineral may be of great assistance in discovering new sites of mineral deposits. Finally, this work lends additional support to the practice of using microbes in mineral extraction.

SUMMARY OF WORK ACCOMPLISHED UNDER THE 1962-63 [redacted] FROM [redacted]

(a) Microbial Action on Mineral Sulfides

Quantitative studies on the action of the Thiobacillus-Ferrobacillus group of bacteria on the minerals orpiment (As_2S_3), arsenopyrite (FeS_2FeAs_2), and enargite ($3Cu_2SAs_2S_5$), were initiated. The bacteria achieved significant solubilization of orpiment, releasing arsenic as arsenite and arsenate. Oxidation of the sulfur portion of orpiment is probable, but remains to be experimentally verified. Some spontaneous, nonbiological oxidation of orpiment occurred, but it was only about one-third as extensive as with bacteria. The chemistry of bacterial oxidation of orpiment appears to differ significantly from nonbiological oxidation as reflected by pH changes during the processes. With bacteria the pH fell from 3.5 to 2.0 in thirty-five days, but without bacteria it rose from 3.5 to 5.0 in that time. The precise chemical mechanism of orpiment oxidation remains to be worked out.

Quantitative work on bacterial oxidation of arsenopyrite showed release of iron, arsenic, and probably sulfur, from the mineral. Most of the analyses in this study were carried out by an undergraduate student for his senior thesis. The results have shown that, contrary to a sustained release of soluble arsenic from orpiment by bacteria, only a limited amount of soluble arsenic was released by them from arsenopyrite. This happened in spite of a pronounced release of iron. The reason for limited release of soluble arsenic by bacteria from arsenopyrite is the precipitation of iron arsenites and arsenates after a critical concentration of soluble iron and arsenite and arsenate has been reached. Although some oxidation of arsenopyrite occurred in the absence of bacteria, its extent was less and its chemistry different, because iron arsenates and arsenites were not precipitated without bacteria, and the pH of the medium dropped from 3.5 to 2.5 in thirty-six days with bacteria and rose from 3.5 to 4.0 in that time without bacteria.

The extensive reprecipitation of iron and arsenic through bacterial action has direct implications with respect to possible translocation of the constituents of arsenopyrites in nature. The bacterial phenomenon also poses a problem when arsenic and iron are to be extracted together from a natural mineral deposit.

Quantitative work on bacterial oxidation of enargite has given results resembling those with arsenopyrite. Although the bacteria are evidently acting on the mineral, a sustained solubilization of the component arsenic is not noted. Indeed, in the presence of bacteria the dissolved arsenic concentration drops after an initial rise. In the absence of bacteria the arsenic concentration rises slightly but continually. It is not clear from the results so far what the fate of the arsenic or copper is after bacterial action because no recognizable precipitate was formed. Contaminating iron is released extensively by bacteria from the mineral but not without them. The released iron appears to remain in the ferrous state. The pH changes are from 3.5 to 2.5 with bacteria, and from 3.5 to 4.5 without bacteria, in thirty-six days. A precise description of the chemical changes that enargite undergoes remains to be worked out.

Growth on cuprous sulfide by the Thiobacillus-Ferrobacillus group, investigated chiefly by a graduate student, is showing variable responses on different synthetic preparations. Bacterial enrichment cultures derived from several mine effluents during the past year have, however, given more consistent growth responses on these preparations. The possibility arises that consistent action on cuprous sulfide requires the participation of more than one organism. In this connection, the principal investigator found protozoa and fungi accompanying the Thiobacillus-Ferrobacillus group of bacteria in mine water from [redacted]. The protozoa included an amoeba and a flagellate, which appeared to grow at the expense of the bacteria and fungi. The amoeba were observed to ingest such organisms.

Both kinds of protozoa are unusual in their tolerance of 800 ppm copper and upwards of 2000 ppm iron. Ordinarily these metal concentrations would be expected to be lethal. The flagellate has been repeatedly subcultured in an iron-salts medium in mixed culture with the Thiobacillus-Ferrobacillus group of bacteria.

(b) Manganese Nodules

During three cruises in the Atlantic Ocean in June, July, and August, 1962, as guest of the [redacted] thirty-four manganiferous samples and twenty-four cores of bottom deposits were taken. About two-thirds of the manganiferous samples have now been tested for their bacterial content and for the ability of these bacteria in aiding manganese addition to the respective samples, as previously described by [redacted] Most of these samples were also analyzed for total iron and manganese content. The results showed that the various manganiferous samples were by no means alike. In some cases this was obvious by visual inspection. In other cases, however, the differences were not macroscopically apparent. Instead, the iron-manganese content, or the bacterial content, or the Mn adsorptive power of the samples, showed differences. Results of experiments, designed to test for the enhancement of Mn adsorption by manganiferous material with the help of the native bacterial flora, could be divided into four major categories. In one, bacterial enhancement of Mn adsorption occurred with or without prior surface-sterilization. In a second, it occurred only with surface sterilization, and in a third only without it. In a fourth, bacterial enhancement of Mn adsorption was not noted under either condition. Since not all bacteria from Mn nodules can enhance Mn adsorption, inclusion of surface-sterilization in the procedure causes selection of different types of organisms from the nodule flora than omission of surface-sterilization. However, the full explanation for the variability of bacterial enhancement of Mn adsorption is more complicated than that. With a number of

samples it was noted that Mn uptake by manganiferous material resulted in a net loss of manganese from the adsorber. The reaction accounting for this phenomenon is the result of a dismutation between Mn^{2+} and MnO_2 and will be discussed further below. This reaction was exhibited by about one-sixth of the samples tested and shows that the properties of these samples are different from the rest. It can therefore be concluded that chemical and physical properties of manganiferous materials help to determine the effectiveness of bacteria in enhancing Mn adsorption. Moreover, since all but one of the samples of the 1962 collection came from extinct submarine volcanoes (sea mounts), and since quite a few of these samples behaved differently from earlier samples from other sources, it is postulated that geographic location of manganiferous samples may affect their chemical, physical, and biological properties.

A clue to the nature of nonbiological Mn release from manganiferous samples is provided by an experiment which shows a dismutation reaction between Mn^{2+} and MnO_2 forming Mn^{3+} when nodules adsorb Mn^{2+}, on the condition that Mn^{3+} is stabilized so as to prevent it from undergoing the reverse reaction. Pyrophosphate was found to be an effective stabilizer. Since pyrophosphate does not occur in a marine environment, protein or amino acids may serve instead as stabilizers. Nodule breakdown can thus be accomplished biologically with sugar as the reducing agent of MnO_2,[2] or nonbiologically by a dismutation reaction between Mn^{2+} and MnO_2. Why the latter reaction occurs with only some nodules, even though all have MnO_2 and adsorb Mn^{2+}, remains to be explained.

The ability of Arthrobacter to aid in Mn addition to nodular substance was shown in pure culture experiments. From similar tests it could be concluded that Vibrio can similarly aid in Mn addition, but not Achromobacter. All three organisms have been found in nodules. Arthrobacter, at high peptone concentrations, causes clumping and heaping of nodular material; not so at low peptone concentrations. A similar phenomenon has been noted in soil . The clumping and heaping

seems to reduce surface area, and therefore slows adsorption of Mn.

Initial experiments have revealed that Arthrobacter can aid in Mn adsorption by "synthetic" MnO_2. However, the adsorption and incorporation process of Mn^{2+} is not permanent. The previously cited dismutation reaction seems to come into play eventually, probably when the bacteria become physiologically inactive at the end of their growth cycle. "Synthetic" MnO_2 is a much poorer adsorbent than nodule material. It is postulated that the iron in nodule material helps to stabilize the manganese in it and, for as yet unknown reasons, increases its adsorptive power.

The core samples of bottom deposits, collected last summer, were examined by enrichment for bacterial content. The distribution of bacteria in these cores was not uniform, nor were the bacteria necessarily of the same kind at different depths in the same core. These findings are similar to those cited by Zo Bell[4]. Work is presently under way to test the ability of the core samples to adsorb Mn with and without bacterial growth. Differences in Mn adsorptive capacity seem to exist among cores on the basis of tests so far. Such differences, if corroborated by further tests, must reflect upon Mn distribution in the sea and upon nodule distribution and structure.

REFERENCES

1. de Grys, Ann. Econ. Geol. 57: 1031-1044 (1962).

[redacted]

3. Martin, J.P., and S.A. Waksman. Soil Science 50: 29-47 (1940).

4. Zo Bell, C.E. MARINE MICROBIOLOGY. Chronica Botanica Co., Publ. 1946, p. 91.

PROGRAM OF FUTURE WORK

(a) Mineral Sulfides

Although it is the intention to continue testing other mineral sulfides for susceptibility to attack by the Thiobacillus-Ferrobacillus group, special attention will be paid to the details of bacterial action on orpiment, arsenopyrite, and enargite. Quantitative methods have to be developed for determining the proportions of various forms of a given element dissolved and reprecipitated after bacterial action. Such information should provide clues to the mechanism of action by the bacteria. The detailed information on the fate of arsenopyrite and enargite, when available, will provide information concerning the effect of side reactions on the overall process of bacterial mineral oxidation. Ultimately, it is hoped to develop methods of mass culture of a desired organism on a particular mineral to get enough cell material for resting cell studies and enzymatic investigations.

(b) Manganese Nodules

Two aims will be followed experimentally in this work. One is the further elucidation of environmental relationships to the process of nodule development or degradation. The second is the elucidation of the biochemical mechanism whereby Mn is added to, or released from, manganese nodules by bacteria. To attain the first aim, samples from various known scurces will be studied in respect to physical, chemical, and biological properties, and attempts will be made to correlate the results with geographic location. In studying the manganese fixing capacity of bottom muds, possible correlation with nodule development will be sought. To attain the second aim, further studies with purified cultures from nodules will be made on nodule material and on synthetic MnO_2 to compare and contrast behavior. Since previous experiments have already shown a difference in action by bacteria between nodule material and MnO_2, a condition is provided to compare the effect of iron on the two systems. It is planned to develop more specific assay procedures for

differentiating the various oxidation states of Mn and thereby to follow the exact fate of adsorbed or desorbed Mn, and to establish more clearly the role bacteria play in this process. The ultimate goal is to study the bacterial action on manganese on an enzymic level.

PUBLICATIONS ARISING OUT OF THE WORK UNDER THIS [redacted] FOR 1962-63

(1) [redacted]

(2) [redacted]

(3) [redacted]

(4) [redacted]

(5) It is anticipated that a paper will be written for submission to Appl. Microbiol. on the work with manganese nodules during the last year.

PERSONNEL FOR 1963-64

Principal Investigator: [redacted] Associate Professor of Biology

Technician: [redacted]

Graduate Student: [redacted]

133-27

April 1963

Proposal No. [redacted]
entitled
[redacted]
for renewal of [redacted]
for the period
1 September 1963 - 31 August 1964

COST ESTIMATE

Personnel		
Principal Investigator		
1/8 time-academic year	$1,175	
1/4 time-summer months	785	$1,960
Technician-full time		4,330
Total Personnel Payments		$6,290
Consumable Supplies		
Chemicals	$ 400	
Glassware	200	600
Permanent Equipment		520
Travel and Communication		
To scientific meetings	$ 250	
Telephone	35	285
Publication of Reports		200
Total Direct Cost		$7,895
Indirect Cost		
14% of Total Direct Cost		1,105
TOTAL		$9,000

[redacted]

[redacted]
Director
Research Division

hk

133-28

[redacted]

Date 29 July 1963

MEMORANDUM FOR: C/TSD/FASS

SUBJECT : MKULTRA, Subproject No. 133

Under the authority granted in the memorandum dated [illegible] from the DCI to the DD/A, and the extension of this authority by memoranda, Subproject 133 has been approved, and $9,360.00 of the over-all Project MKULTRA funds has been obligated to subproject's expenses and should be charged to cost center 4125-1390-3902

[redacted]

Concur:

Asst. Chief, TSD for R&D

Concur:

APPROVED FOR OBLIGATION
OF FUNDS:

Chief, DD/P/TSD

Date:

Distribution:
Orig & 1 - Addressee
2 - TSD/

[redacted]

[redacted]

[redacted] REVISED 133-29

Date 18 February 1963

Branch EB Category Biological Energy Systems (IVc)

Project Title Microbial Transformation of Minerals Item Classification Unclassified (Reports)

Project Crypto MKULTRA Sub. # 133 Crypto Classification Unclassified

Branch Project No. N.A. Project Engineer [redacted] A

Contractor [redacted] C

Contract No. N.A. Task No. N.A.

Type of Contract MKULTRA Date Initiated 12 July 1961

Cost $9,000.00 Completion Date Continuing

Purpose: To provide the services of [redacted] C and to support research on fundamental mechanisms of mineral transformation by microorganisms. In addition to the direct potential for new energy source systems, the contract may provide by-products useful for material deterioration application.

Status: Current and satisfactory.

SOURCE: Internally generated in support of the requirement to seek new and better sources of energy for battery application.

[redacted]

[redacted]

133-30

I hereby acknowledge receipt of the following:

[redacted] Check No. 0069284, dated Oct 11, 1962, drawn on the [redacted] payable to [redacted] in the amount of $9,000.00.

[redacted] Check No. 0069286, dated Oct 11, 1962, drawn on the [redacted] payable to [redacted] in the amount of $360.00

[redacted]

Date: 10-16-62

REQUEST FOR ADVANCE OF FUNDS

NAME [redacted]	DATE 5 October 1962	AMOUNT 9,988.00 (Fee)	VOUCHER NO. *(Finance use only)*
PAYABLE TO See attached.	ROOM [redacted] BUILDING	TELEPHONE EXT.	

132-31

PURPOSE

To [redacted] for funding MKULTRA Subproject 133 Invoice #2 which activity was approved by C/TSD on 25 July 1962. Accounting to be in accordance with the Fiscal Annex Attachment C.

STATUS OF OUTSTANDING ADVANCES

DATE	AMOUNT	UNACCOUNTED BALANCE

I agree that I will fully account for this advance by submission of vouchers and refund of any unexpended balance to the reporting point stated and by the due date checked below. In the event of my failure to so account and refund any unexpended balance, I authorize deduction from my salary to effect settlement.

REPORTING POINT	DUE DATE	✓
FINANCE DIVISION - HEADQUARTERS		
	ON ARRIVAL AT DESTINATION ON OR ABOUT	
	MONTHLY - ON THE LAST WORKDAY OF EACH MONTH	

REQUESTING OFFICER

DATE | SIGNATURE [redacted]

APPROVED

DATE | SIGNATURE OF APPROVING OFFICER: Sidney Gottlieb, DC/TSD

RECEIPT FOR FUNDS ADVANCED

I acknowledge receipt of funds in the amount stated hereunder to be used for the purpose stated and accounted for as shown above.

DATE | AMOUNT

I CERTIFY FUNDS ARE AVAILABLE

OBLIGATION REFERENCE NO.: 176 | CHARGE ALLOTMENT NO.: 3125-1390-3902

CERTIFIED FOR PAYMENT OR CREDIT

DATE | AUTHORIZED CERTIFYING OFFICER

DATE | SIGNATURE

SIGNATURE OF ADVANCEE

SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION

PREPARED BY | REVIEWED BY | VOUCHER NO.: 7-12

DESCRIPTION-ADVANCE ACCOUNTS 13-27	DESCRIPTION-ALL OTHER ACCOUNTS 13-33	28-33 T/A NO. / P.O. NO. / 32-33 DIV.	34-39 STATION CODE / PROP. NO. / PROJECT NO.	40-42 EXPEND CODE / FY	43 FUNDS	45-46 PAY PERIOD CODE	47-52 OBLIG. REF. NO. / ADVANCE ACCT. NO. / EMP. NO.	53 CA YR	54-57 GENERAL LEDGER ACCT. NO.	58-67 ALLOT. OR COST ACCT. NO.	62-67 CK. NO. / X REF. NO.	68-70 DUE DATE / OBJECT CLASS	71-80 AMOUNT DEBIT	71-80 AMOUNT CREDIT
											TOTALS			

FORM 5-60 281 USE PREVIOUS EDITIONS. (38-49)

133-32

[redacted]

Date: 24 July 1962

MEMORANDUM FOR: The Comptroller

ATTENTION : Finance Division

SUBJECT : MKULTRA, Subproject 133

Under the authority granted in the memorandum dated 13 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 133 has been approved and $9,360.00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 3125-1390-3902

A [redacted]

TSD/Biological Branch

APPROVED FOR OBLIGATION OF FUNDS:

XXXXXXXXXXXXXXXXX
Acting Chief, TSD

Date: July 26, 1962

Distribution:
Original & 2 - Addressee

CERTIFY THAT FUNDS ARE
OBLIGATION REFERENCE No.
CHARGE TO [redacted] 0-3902

A

[redacted]

[redacted]

133-33

24 JUL 1962

DRAFT

MEMORANDUM FOR: THE RECORD

SUBJECT : Continuation of MKULTRA, Subproject 133

1. The purpose of MKULTRA, Subproject 133 is to enable TSD/BB to utilize the services of [redacted] C B

[redacted] B

2. During the first year of the program [redacted] C has made significant contributions to the all too scanty knowledge of the mechanisms of mineral transformations. A technical discussion of these accomplishments is attached hereto, with an outline of proposed work for the coming year. It is possible that these investigations may well lead to new approaches for energy transfer systems (bio-batteries) and deterioration of metals.

3. [redacted] B functioned as [redacted] B and [redacted] B during the first year of this project. This service will heretofore be furnished by the [redacted] B The cost of this program for the second year will be $9,000.00 to which must be added $360.00 which represents a 4% service charge to be retained by the [redacted] B The total cost of the program, therefore, will not exceed $9,360.00. Charges should be made against Allotment No. 3125-1390-3902.

4. It is not anticipated that permanent equipment other than that listed in the budget will be required for this program. Title to the

[redacted]

[redacted]

equipment listed will be retained by the [redacted] in lieu of higher overhead rates.

5. Documentation and accounting for travel expenses which are reimbursable by [redacted] will conform to the accepted practice of that organization.

6. [redacted] has been cleared COVERTLY and is unwitting and will remain unwitting of the true nature of the sponsor.

[redacted]
TSD/BIOLOGICAL BRANCH

[redacted]
CHIEF
TSD/BIOLOGICAL BRANCH

APPROVED FOR OBLIGATION OF FUNDS:

_____
AC/TSD

DATE: _____

Attached:
Budget
Project Summary and Proposal

Distribution:
Original only

[redacted]

133-33

[redacted]

25 June 1962

[redacted]

Attention: [redacted]

Subject: Proposal entitled [redacted]

Please find enclosed one copy of the subject proposal submitted on behalf of [redacted]

The proposal is not being submitted elsewhere for possible support.

Your consideration of our proposal will be appreciated and we look forward to hearing from you.

Very truly yours,

[redacted]

Assistant Director

[redacted]

Enclosure

133-33

[redacted]

Proposal entitled

[redacted]

Submitted on behalf of

[redacted]

Associate Professor of Biology

June 1962

133-33

DEPARTMENT OF BIOLOGY

[redacted]

June 14, 1962

[redacted]

Purpose of Study:

The purpose of this proposal is a request for financial support to continue an investigation of microbial action on marine manganese nodules and terrigenous mineral sulfides, which the principal investigator has been pursuing since 1958. Very intensive work on these materials is being carried on by him, with fruitful results, during the current year, 1961-62, under a [redacted] from the [redacted] Since relatively little is known about microbial mineral transformation, and in view of current academic and practical interest of microbiologists, geologists, mining engineers, soil scientists, oceanographers, etc., in the subject, this research should make a valuable contribution to science.

Summary of Past Work:

a. Bacteriology of mineral sulfides.

Attempts were made to evaluate the microbial flora isolable from unsterilized, crushed sulfide minerals by enrichment in mineral solution. The following minerals were studied: alabandite, arsenopyrite, bornite, chalcocite, chalcopyrite, cinnabar, cobaltite, covellite, enargite, galena, marcasite, orpiment, pyrite, pyrrhotite, realgar, and sphalerite. Of these minerals, only cobaltite, enargite, galena, pyrite, pyrrhotite, realgar, and sphalerite yielded microorganisms. For the most part these organisms were heterotrophic and probably represented contaminants. However, Hyphomicrobium, isolated from realgar, a pink yeast repeatedly isolated

from sphalerite, and Arthrobicter, isolated from cobaltite, galena, pyrrhotite, realgar, and sphalerite may constitute part of a normal flora. The action of any of these organisms with respect to the mineral with which they were found associated remains to be established.

After surface-sterilization, some of the above mineral sulfides, when enriched in mineral solution, have yielded iron-oxidizing autotrophs. These minerals include arsenopyrite, pyrite, pyrrhotite, chalcopyrite, enargite, galena, marcasite, and sphalerite. At least some of the isolated bacterial strains are not restricted to a diet of iron for energy, but can use sulfur or, probably, some other oxidizable metals.

The ability to grow on any of the above sulfide minerals was tested by inoculating surface-sterile samples in oxidizing columns with Ferrobacillus ferrooxidans, and attempting to recover the organism from effluent feeding solution over a period of two months or more. So far, positive results have been obtained with arsenopyrite, enargite, chalcopyrite, marcasite, galena, pyrite, pyrrhotite, and sphalerite. Negative results have been obtained with alabandite, bornite, cobaltite, covellite, chalcocite, and one sample of galena. Cinnabar, orpiment, and realgar are being currently investigated.

In addition to the foregoing qualitative work, quantitative studies on the rates of oxidation of synthetic Cu_2S and natural arsenopyrite are presently being undertaken. From these studies it has become clear that synthetic Cu_2S can be oxidized at least 4x as fast by bacteria than by autoxidation, and that arsenopyrite can be more rapidly oxidized by bacteria than by autoxidation. Results with the latter material are not yet sufficient to establish an exact rate comparison. The precise mech-

anism of bacterial oxidation remains to be established. The work with synthetic Cu_2S proves, what some other workers seem to doubt, that F. ferrooxidans can oxidize metals other than iron.

b. Manganese Nodules

Oceanographers have felt pretty strongly in the past that the origin and development of manganese nodules in the oceans is attributable to purely physicochemical processes. However, [redacted] on finding organic nitrogen in nodules, concluded that biological agents were involved in nodule genesis. At his suggestion, the principal investigator attempted to find out if bacteria might play a role in this. He found that bacteria were indeed present in the nodular substance after surface-sterilization (a rough estimate at present is 10^4 per gram). They included Achromobacter, Arthrobacter, Bacillus, Brevibacterium, Staphylococcus, Vibrio, an unidentified rod, and an unidentified coccus. The principal investigator showed in quantitative experiments that nodular substance can adsorb manganous ion from sea water, and that this adsorption is accelerated by bacteria that grow from the nodular material. The acceleration of manganous ion adsorption is explainable on the basis that the bacteria oxidize the adsorbed manganese, which facilitates further adsorption of manganous manganese. The acceleration requires the presence of peptone, to permit bacterial development. If peptone and glucose are present, manganese is released from the nodular substance rather than adsorbed, at least in a net effect. Since some nodules were apparently initiated around shark's teeth, ear bones of whales, pumice, etc., in the sea, attempts were made to see if oyster shells can adsorb manganous manganese and thus serve as possible foci of nodules.

It was found that they do adsorb it and that peptone did not stimulate this adsorption (no bacteria were present!). As far as a survey of the literature has gone, these observations with respect to manganese nodules have not been reported before.

Pertinent literature:

The early literature dealing with microbial action on minerals has been covered by Alexander (1). A review by Lyalikova summarizes much of the past important work on Thiobacillus ferrooxidans and Ferrobacillus ferrooxidans (2). An intimate association of iron-oxidizing autotrophs with natural mineral sulfides has been indicated by the work of [redacted] and by that of Lyalikova (5). Differences of opinion exist between Bryner and Anderson (6), Malouf and Prater (7), and Ivanov, Nargirvyak, and Stepanov on the one hand, and [redacted] (8) on the other about a mechanism of mineral sulfide oxidation of chalcopyrite, molybdenite, chalcocite, and sphalerite, for instance. No previous studies on bacteria in manganese nodules has been reported. However, bacterial manganese oxidation and reduction by soil bacteria has been known for some time. An important quantitative study on large-scale bacterial manganese metabolism is that of Mann and Quastel (9). Descriptions of manganese nodules are given by Murray (10) and Dietz (11). A chemical and physical study of nodules was made by Buser and Gruetter (12). The finding of organic nitrogen in nodules was first reported by Graham (13) and Graham and Cooper (14), who also suggested a biological origin of the nodules on this basis.

References:

1. Alexander, M., INTRODUCTION TO SOIL MICROBIOLOGY, John Wiley & Sons Co., 1961.

2. Lyalikova, N. N., Mikrobiologiya 29: 773-779 (1960).

[redacted]

5. Lyalikova, N. N., Mikrobiologiya, 30: 135-139 (1961).

6. Bryner, L. C., and R. Anderson, Ind. Eng. Chem. 49: 1721-1724 (1957).

7. Malouf, E. E., and J. D.Prater, J. Metals 13: 353-356 (1959).

8. Razzell, W. E., Annual Western Meeting, Vancouver, Oct. 1960 Transactions, LXV, 1962 pp. 135-136.

9. Mann, G., and J. H. Quastel, Nature 158: 154-156 (1946).

10. Murray, J., VOYAGE OF H.M.S. CHALLENGER. DEEP SEA DEPOSITS. Her Majesty's Stationary Office. 1891.

11. Dietz, R. S., J. Calif. Mines and Geol. 51: 209-220, (1955).

12. Buser, W. and A. Gruetter, Schweiz. mineralog. petrogr. Mitt. 36: 49-62, (1956).

13. Graham, J. W., Science 129: 1428-1429 (1959).

14. Graham, J. W. and Susan Cooper, Nature 183: 1050-1051.

Pertinent Publications by Principal Investigator:

[redacted]

[redacted]

Proposed New Work:

Continuation of present lines of investigation:

a. Mineral Sulfides:
   1. Continuation of survey of natural sulfide minerals for a normal flora, with particular emphasis on large-scale microbial action on minerals.
   2. Characterization of isolates (physiological and morphological).
   3. Examination of isolates for specific mineralizing activities.
   4. Elucidation of biochemical mechanisms of mineral transformation.

b. Manganese Nodules:
   1. Qualitative and quantitative bacteriological comparison of manganese nodules from different oceans.
   2. Study of the biochemical mechanism of manganous oxidation and MnO_2 reduction in the various bacteria isolated.
   3. Determination of the mechanism of iron-incorporation into manganese nodules.

The methods to be used in these studies will be adaptations of standard procedures of bacteriology, physiology, and biochemistry.

Personnel:

Principal Investigator: [redacted] Assoc. Prof. of Biology.

Technician: [redacted]

Graduate Student: [redacted] (not presently supported)

Undergraduate student: [redacted] undergraduate research fellow) [redacted] (summer 1962) (summer 1963)

Proposed Budget:

PERSONNEL		
Principal Investigator		
1/8 time-academic year	$ 1,050	
1/4 time-summer months	700	
Technician-full time	4,155	
		$ 5,905
PERMANENT EQUIPMENT		
Fluorimeter		900
CONSUMABLE SUPPLIES		
Chemicals	$ 300	
Glassware	340	
		640
TRAVEL		
To scientific meetings		250
OTHER EXPENSES		
Publication	$ 180	
Telephone	20	
		200
Total Direct Cost		$ 7,895
INDIRECT COST		
@ 14% of Total Direct Cost		1,105
Total Cost		$ 9,000

133-34

[redacted]

File 63

Date 24 July 1962

Branch BB Category Biological Energy Systems (IVc)

Project Title Microbial Transformation of Minerals Item Classification Unclassified (Reports)

Project Crypto MKULTRA, Sub #133 Crypto Classification Unclassified

Branch Project No. N.A. Project Engineer [redacted] A

Contractor [redacted] B/C

Contract No. N.A. Task No. N.A.

Type of Contract MKULTRA Date Initiated 12 July 1961

Cost $9000.00 Completion Date Continuing

Purpose: To provide the services of [redacted] C and to support research on fundamental mechanisms of mineral transformation by microorganisms.

Status: The first year of this project is judged satisfactory in all respects and a summary report has been received [redacted]

133-35

14 August 1961

[redacted]

Gentlemen:

We are pleased to be able to transmit to you the following funds:

[redacted] No. 268654 drawn on the [redacted] for $360.00.

Cashier's Check No. 2-064674 drawn on the [redacted] for $9,000.00

These funds represent a contribution for the use of your directors in carrying out the very worthwhile research goals of your organization.

Yours truly,

Enclosures (2)

133-36

[redacted]

12 July 1961

MEMORANDUM FOR: Chief, Finance Division

VIA : TSD/Budget Officer

SUBJECT : MKULTRA, Subproject 133, Invoice #1
Allotment 2125-1390-3902

1. Invoice No. 1 covering the above subproject is attached. Payment should be made as follows:

Cashier's check in the amount of $9,000.00 drawn on a [redacted] payable to [redacted] B

Cashier's check in the amount of $360.00 drawn on a [redacted] payable to [redacted] B

2. Please forward the checks to Chief, TSD/Research Branch, through TSD/Budget Officer, no later than 24 July 1961.

3. This is a final invoice. However, since it is anticipated that additional funds will be obligated for this project, the files should not be closed.

[redacted] A
Chief
TSD/Research Branch

Attached:
Invoice & Certifications

Distribution:
Orig & 2 - Addressee
1 - TSD/FASS
2 - TSD/RB

TSD/RB [redacted] A

[redacted]

133-36

[redacted]

INVOICE

For Services $9,360.00

[redacted] B.

CERTIFICATIONS

(1) It is hereby certified that this is Invoice #1 applying to sub-project No. 133 of MKULTRA, that performance is satisfactory, that services are being accomplished in accordance with mutual agreements, that a detailed agenda of the payments and receipts is on file in TSD/RB, that this bill is just and correct and that payment thereof has not yet been made.

Chief, TSD/Research Branch

Date:

(2) It is hereby certified that this invoice applies to SubProject 133 of MKULTRA which was duly approved, and that the project is being carried out in accordance with the memorandum of 13 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda.

Research Director

Date:

[redacted]

133-37

[redacted]

DATE: 25 May 1961

MEMORANDUM FOR : THE COMPTROLLER

ATTENTION : Finance Division

SUBJECT : MKULTRA, Subproject 133

Under the authority granted in the memorandum dated 13 April 1953 from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 133 has been approved, and $9,300.00 of the over-all Project MKULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 1135-130-3XX.

[redacted]
Chief
TSD/Research Branch

APPROVED FOR OBLIGATION OF FUNDS:

Research Director

Date:

Distribution:
Orig. & 2 - Addressee
1 - TSD/FASS
2 - TSD/RB (1 chron, 1 file(finance)

[redacted]

133-38

[redacted]

DRAFT [redacted]

MEMORANDUM FOR: The Record

SUBJECT : Initiation of MKULTRA, Subproject 133

1. The purpose of MKULTRA, Subproject 133 is to enable TSD/RB to utilize the services of [redacted]

2. [redacted] will conduct a program of research on mineral transforming microorganisms. The work will consider both ecological and physiological aspects of the problem. Ways may also be shown for growing food from minerals by a non-photosynthetic process because some bacteria, already known to attack certain minerals, can change carbon dioxide into organic matter with the energy that they derive from the oxidation of minerals.

3. [redacted] shall function as [redacted] and [redacted] for this project. The cost of this program for the first year will be $9,000.00 to which must be added $360.00 which represents a 4% service charge to be retained by the [redacted]. The total cost of the program, therefore will not exceed $9,360.00. Charges should be made against Allotment No. 2125-1390-3902.

4. It is not anticipated that any permanent equipment will be required for this program.

5. Documentation and accounting for travel expenses which are reimbursable by [redacted] will conform to the accepted practice of that organization.

[redacted]

C

6. [redacted] has been cleared COVERTLY and is unwitting and will remain unwitting of the true nature of the sponsor.

A [redacted]
TSD/Research Branch

A [redacted]
Chief
TSD/Research Branch

APPROVED FOR OBLIGATION
OF FUNDS:

A [redacted]
Research Director
12 1961

Date:

Attached:
Budget
Proposal & Literature Review

Distribution:
Original only

[redacted]

133-38

[redacted]

BUDGET

1.	Salaries	
	Principal Investigator	$4,500.00
	Assistant	$4,000.00
2.	Travel	$ 200.00
3.	Supplies	$ 300.00
	TOTAL	$9,000.00

[redacted]

A STUDY OF MINERAL-TRANSFORMING MICROORGANISMS

Purpose:

The project proposed herein is intended to provide information on the microbiological participation in mineral transformation. Only scanty knowledge is available in this field at the present time. The work will consider both ecological and physiological aspects of the problem. The findings should be a valuable contribution to fundamental knowledge in microbiology, geology, and allied fields. They should also be a valuable contribution to practical knowledge in these areas because ways may be shown to harness some of these microorganisms to do useful work for Man, such as aiding in the conversion of minerals of low economic value into substances of high economic value. Ways may also be shown for growing food from minerals by a non-photosynthetic process, because some bacteria, already known to attack certain minerals, can charge CO_2 into organic matter with the energy which they derive from the oxidation of the minerals, (1). This organic matter may then in turn serve as food for other microorganisms which may be used directly or indirectly as food for Man.

From preliminary investigations by the senior investigator (2), it has become apparent that mineral-transforming microorganisms are intimately associated with various types of minerals. The proposed research is designed to follow up the leads from this earlier work to delineate clearly the environmental and functional

interrelationships between mineral habitats and the microorganisms found therein.

Objectives:

a) Isolation of representative organisms by enrichment and pure culture methods:
   1. from ores and other minerals
   2. from drainage waters of mineral deposits
   3. from soils

b) A study of the over-all chemical activity on various mineral substances by enrichment cultures and pure cultures. The mineral substances will include
   1. natural minerals
   2. synthetic minerals

c) A study of the mechanisms of action on various mineral substances by microbial isolates.

Plan of Research:

a) Isolation of representative cultures by enrichment and pure culture methods.

   Microbial enrichments will be derived from crushed ores, other mineral substances, and soils by percolating nutrient salt solutions through percolation columns charged with these materials. The effluent solutions will be subcultured in appropriate selective media, such as iron broth (3), sulfur broth (4), thiosulfate broth (4), nutrient broth, etc.

Microbial enrichments will also be derived from flask cultures prepared by overlaying crushed ore, other mineral substances, and soils with a nutrient salt solution. Microbial enrichments of drainage waters from mineral deposits will be made by inoculating suitable volumes into the various selective media already cited. Other enrichment methods may be used as they become appropriate. The ores and other mineral substances will be used before and after surface sterilization.

Pure cultures from the various enrichments will be prepared by streaking on solidified inorganic and organic media, whenever possible. In cases where neither highly purified agar or silica gels work, a dilution technique using selective liquid media may be employed. Culture purity will be established by appropriate morphological and cultural observations. Attempts at establishing the taxonomic identity of the isolates will be made.

b) A study of the over-all chemical activity on various mineral substances by enrichment cultures and pure cultures.

The over-all chemical activity will be studied with enrichment cultures and pure cultures acting on mineral substances of known composition. In these studies, qualitative and quantitative measurements will be made on substrate decomposition, product accumulation, changes in titratable acidity, pH, redox potential, consumption of CO_2, O_2, and organic matter, if any. A comparison

of activities in enrichment cultures with activities in pure cultures on identical media will reveal whether the cooperation of more than one microorganism is required for a given transformation.

c) A study of the mechanisms of action on various mineral substances by microbial isolates.

The mechanism of action on various mineral substrates by pure cultures will be investigated by the use of standard chemical, biochemical, and physiological techniques. The experimental approach will include a quantitative analysis of the action on pure mineral compounds, on soluble forms of the cations and anions of which the respective minerals are composed, and on the theoretically possible intermediates of metabolism. Such information will be required for the formulation of a scheme that would explain the possible over-all chemical reaction observed.

References:

1. Silverman and Lundgren, J. Bacteriol. 78: 326 (1959).

[redacted]

3. Silverman and Lundgren, J. Bacteriol. 77: 642 (1959).

4. MANUAL OF MICROBIOLOGICAL METHODS, McGraw-Hill Book Co., Inc., New York, 1957, p. 114.

[redacted]

[redacted]

8. [redacted]

Literature Review:

A number of reports exist in the literature about microorganisms which seem to be associated with processes of mineral transformation. In many instances, the function which these microorganisms play is not known or has been misinterpreted. The following is a discussion of some reports on microbial action on minerals common in nature.

Sulfur:

An inspection of the 7th edition of Bergey's Manual of Determinative Bacteriology reveals that bacteria have been associated in the past with sulfur oxidations and reductions (1,2). The Thiobacteriaceae include members that have been shown to oxidize various forms of sulfur more reduced than sulfate. These organisms are all obligately or facultatively chemoautotrophic. Most are strictly aerobic, except for Thiobacillus denitrificans, which is facultative. Photosynthetic bacteria, belonging to the Chlorobacteriaceae and the Thiorhodaceae, have been shown to use inorganic sulfur compounds other than sulfate as reductants in photosynthesis. More questionable is the association of higher bacteria, the Beggiatoaceae and Achromataceae, with sulfur oxidation. Their association with sulfur is largely based on the observation of intracellular deposition of sulfur granules. Faust and Wolfe have recently shown that Beggiatoa alba's activity on sulfur is restricted if not absent, at least in laboratory culture (3). The habitats of all these bacteria seem to be soil, fresh and marine waters, and sulfur deposits (4). In at least one instance, a sulfur oxidizer has been isolated from coprolite rock (fossil dung) of the Triassic period (1). A fairly recent review of the sulfur bacteria is contained in (5).

Sulfur reduction has been most extensively studied in relation to the activities of Desulfovibrio desulfuricans of

the Spirillaceae. Members of this genus reduce sulfates, thiosulfates, sulfites, etc. to hydrogen sulfide under anaerobic conditions. The habitats of these bacteria include soil, sewage, water, oil-bearing strata, etc. Uptodate discussions or the physiology or these bacteria are contained in (6,7).

Iron compounds:

The interaction or bacteria with iron compounds was observed as early as 1888 (8). In this work, a sheathed bacterium, Leptothrix, was observed to deposit ferric hydroxide in its sheath. It was believed that the organism was chemoautotrophic, getting its energy for carbon dioxide assimilation from the oxidation of ferrous iron. However, the chemoautotrophic nature or this organism and many other iron bacteria has not been satisfactorily proven in the light of our present understanding of chemoautotrophy. At present it seems more likely that the sheathed bacteria (Chlamydobacteriaceae, Crenotrichaceae) deposit ferric hydroxide from ferruginous waters in their sheaths without metabolic mediation. The same kind of ferric hydroxide deposition is believed to take place in the case of the stalked bacteria (Caulobacteriaceae). In their case, too, it is at present questioned whether chemoautotrophy accounts for the ferric hydroxide deposition. The one exception in this instance may be Gallionella ferruginea (9). Among encapsulated bacteria (Siderocapsaceae), the majority of the bacteria seem to deposit ferric hydroxide in their capsules or outer cell-surfaces by non-chemoautotrophic means. Only the genus Ferrobacillus in this family oxidizes ferrous iron by strictly chemoautotrophic means, at a very acid pH (10). This genus is probably related to the Thiobacteriaceae (1). The

reported habitat for all these bacteria is iron-bearing water. Certain iron ores also seem to contain them (11).

Various common bacteria are able to precipitate iron from organic salt combination (12). Such genera as Escherichia, Aerobacter, Pseudomonas, Bacillus, etc. are able to bring this about by digesting away the organic portion of the salt. Actinomycetes of the genera Nocardia and Streptomyces can also carry out such activity.

Ferric iron reduction may be promoted directly or indirectly by microbial agents. The indirect effect is probably the more common, taking place in a reducing environment at acid pH created by various bacteria. However, Bacillus circulans can reduce ferric iron through direct metabolic interaction (13). Iron sulfides may be precipitated through the formation of hydrogen sulfide by sulfate reducers or by release of hydrogen sulfide from organic sulfur compounds by various common bacteria.

Manganese compounds: deposition

Manganese has been associated with many of the Siderocapsaceae which are also associated with iron deposition. In general, evidence is lacking for metabolic dependence of this manganese precipitation (1,2), although Beijerinck reported long ago that some true bacteria and fungi from soil could oxidize manganese sulfate or manganese carbonate (heterotrophically) (14,15). Some bacteria seem to be able to reduce manganese dioxide under conditions where this compound can act as an electron acceptor in place of oxygen. Quastel and Scholefield (16) reported on such a process. Chemoautotrophic manganese oxidation has been reported by Prave (17) and by Sartory and Meyer (18), but confirmation of these observations is required.

Copper compounds:

Transformation of copper compounds has been reported by Ciferri and Scaramuzzi (19), Hurwitz (20), and Bryner and Jameson (21). Only in the last of these three reports does evidence exist that enzymatic action on copper compounds is involved; in fact, the bacteria in that report are described as chemoautotrophs allied to the chemoautotrophic iron oxidizers. In the first two reports, metabolic end-products are held responsible for the transformation of insoluble copper compounds.

Molybdenum compounds:

An organism related to the chemoautotrophic iron-oxidizers has been discovered to be able to oxidize the mineral molybdenite (MoS_2) (22). The products of its action on this mineral are sulfate and molybdic acid. Nothing further seems to be known about this transformation.

Silicates:

Bacillus siliceus has been reported to release potassium from a bound state in aluminum silicates when the organism is growing in a potassium deficient environment (23). Aspergillus niger has also been reported to decompose clay minerals to satisfy its potassium requirements (24). Acid formation and its subsequent action on the minerals probably accounts for the potassium release. The acids may include carbonic, organic, nitric, and sulfuric acids.

Conclusion:

This literature review is intended to bring out the fact that microorganisms may bring about mineral transformation in two ways; by direct enzymatic interaction, usually causing oxidation or reduction; or by indirect action through the production of environmental conditions which promote transformations through

the production of environmental conditions which promote transformations through chemical reactions of a purely non-biological nature. It is the first type of transformation which will be stressed in the proposed work.

In spite of the impression that certain problems of microbial mineral transformation have been resolved, more extensive inspection of the pertinent literature reveals contradictions. Three examples bearing on the problem of microbial action in mineral sulfide transformation will be cited for illustration.

Leathen et al. (25) claimed that they could observe Ferrobacillus ferrooxidans to oxidize marcasite but not pyrite. Both these minerals have the same composition (FeS_2). They attributed this difference in action of the bacteria to a difference in crystal structure between marcasite and pyrite. Yet, Bryner et al. (21) and [redacted] could obtain growth on pyrite by F. ferrooxidans or a closely related organism.

A second unresolved problem concerns the oxidation of molybdenite (21). In this work, an organism related to F. ferrooxidans oxidized the mineral to sulfate and molybdic acid. The workers assumed that oxidation of the sulfur components of the mineral accounted for this reaction. They overlooked completely the fact that molybdenum had itself undergone an oxidation. The question thus arises whether the bacteria were directly responsible for molybdenum oxidation.

A third item of conflicting information concerns the reported ability of F. ferrooxidans to grow on media solidified with agar (26). Bryner and Jameson could not confirm this observation (27).

It is thus clear that even on those microorganisms for which extensive information is available concerning their role in mineral transformation, further wrk is needed to clarify various points of conflicting information.

References:

1. Bergey's Manual of Determinative Bacteriology, 7th. ed., The Williams and Wilkins Co., Baltimore, 1957

2. Skerman, V. B. D., A GUIDE TO THE IDENTIFICATION OF THE GENERA OF BACTERIA, The Williams and Wilkins Co., Baltimore, 1959

3. Faust and Wolfe, J. Bacteriol. 81: 99 (1961)

4. Kusnetzov, Bacteriol. Proc., 1961, p.36.

5. Vishniac and Santer, Bacteriol. Rev. 21: 195 (1957)

6. Postgate, Ann. Rev. Microbiol. 13: 505 (1959)

7. Mecnalas and Rittenberg, J. Bacteriol. 80: 501 (1960)

8. Winogradsky, Botan. Zeitung 46: 261 (1888)

9. Vatter and Wolfe, Bacteriol. Proc. 1955, p.35

10. Silverman and Lundgren, J. Bacteriol. 78: 326 (1959)

11. [redacted]

12. Halvorson, Soil Sci. 32: 141 (1931)

13. Bromfield, J. Gen. Microbiol. 11: 1 (1954)

14. Beijerinck, Folia Microbiol. 2:123 (1913)

15. Beijerinck, Verslag Akad. Wetenschappen, 22: 415 (1913)

16. Quastel and Scholefield, Soil Sci. 75: 279 (1953)

17. Prave, Arch Mikrobiol. 27:33 (1957)

18. Sartory and Meyer, Compt. Rend. Acad. Sci. 225: 541 (1947)

19. Ciferri and Scaramuzzi, Atti Ist. Bot. Univ. Lab. Crittog. Pavia, Ser. 5, 3: 233 (1937)

20. Hurwitz, Soil Sci. Soc. Am., Proc. 12: 195 (1947)

21. Bryner and Jameson, Appl. Microbiol. 6: 281 (1958)

22. Bryner, Loren, and Anderson, Ind. Eng. Chem. 49: 1721 (1957)

23. Aleksandrov, and Zak, Mikrobiologiya 19: 99 (1950)

24. Eno and Reuszer, Soil Sci. 80: 499 (1955)

26. Leathen, Braley, and McIntyre, Appl. Microbiol. 1: 65 (1953)

27. Temple and Colmer, J. Bacteriol. 62: 605 (1951)

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